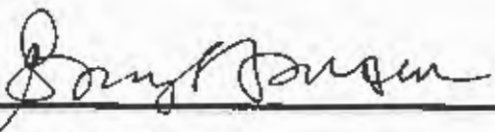


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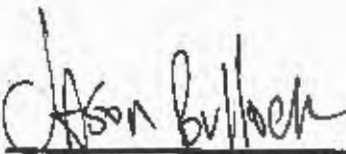
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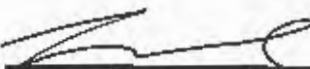
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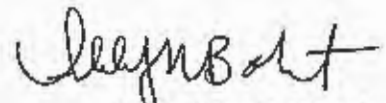
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**Effects of 40% Phosphoric Acid Etch on the Compressive Strength of
Biodentine**

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List of Abbreviations

MPa.....	Mega Pascal (compressive stress at maximum load)
lbf.....	Pounds per Force (maximum load)
mm.....	Millimeter
<i>F</i>	Force
<i>d</i>	Diameter
IRM	Intermediate restorative material
min	Minute
M	Mean
SD	Standard deviation
MTA	Mineral trioxide aggregate
WMTA	White mineral trioxide aggregate
GI	Glass ionomer
RMGI	Resin modified glass ionomer

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Abstract

Objective: This in-vitro study investigated whether an application of 40% phosphoric acid etch significantly alters the compressive strength of Biodentine, a relatively new calcium silicate based dentin replacement material.

Materials and Methods: Compressive strength of Biodentine was determined according to ISO 9917:2010 and ADA specification No. 96 for water based cements. Specimens were prepared using stainless steel cylindrical split molds with the internal dimension of 4.0 ± 0.1 mm in diameter and 6 ± 0.1 mm in length. Specimens were stored in 100% humidity at 37 degrees Celcius for 15 minutes before they were randomly divided into two group—Biodentine subjected to 40% phosphoric acid etch and non-etched Biodentine. The specimens were then loaded to failure in compression using a cross head speed of 30mm/min. Twenty specimens from each group were tested.

Results: The compressive strength at maximum load of the acid-etched samples ($M = 2.6$, $SD = 0.70$) was not significantly different than the non-etched samples ($M = 2.68$, $SD = 0.70$). Additionally, results indicate that the acid-etched sample ($M = 7.39$, $SD = 1.98$) was not significantly different than the non-etched sample ($M = 7.57$, $SD = 1.98$) in terms of the maximum load.

Conclusion: After a time period of 15 minutes, a phosphoric acid etch procedure had no adverse effect on the compressive strength of Biodentine.

Purpose

To determine if phosphoric acid etch significantly alters the compressive strength of Biodentine.

Significance

Compressive strength is considered one of the main physical characteristics of hydraulic cements (Malkondu, Kazandag, & Kazazoglu, 2014). When used as a pulp capping agent or in addition to a composite restoration in the “sandwich” technique, Biodentine™ must be etched with phosphoric acid before placing the composite restoration. Therefore, it is important to determine if there are changes in compressive strength following acid etching. A significant change in the compressive strength of Biodentine™ due to phosphoric acid etching could potentially be a contraindication to using this restorative material in these clinical situations.

Introduction

Biodentine™ is a calcium silicate based dentin replacement material which became commercially available in 2009. According to its manufacturer (Septodont, <http://www.septodontusa.com/>), Biodentine™ has a wide range of applications in both endodontics (e.g. repair of root perforations, apexification, retrograde filling material) and restorative dentistry (e.g. permanent dentin

restoration under composites restoration of cervical radicular lesions, pulpcapping).

Biodentine has many similar properties to mineral trioxide aggregate (MTA), another calcium silicate based material, which has an excellent clinical history (Bajic et al., 2014). Biodentine's lower cost and shorter setting time offer potential advantages over MTA.

Indications for Use

Biodentine™ is a calcium silicate based product which became commercially available in 2009 (Malkondu, Kazandag, & Kazazoglu, 2014). According to its manufacturer (Septodont, <http://www.septodontusa.com/>), the indications for the use of biodentine are as follows:

In the crown:

- permanent dentin restoration under composites or inlay/onlay
- temporary dentin-enamel restoration
- restoration of deep and/or large coronal carious lesions (sandwich technique)
- restoration of cervical radicular lesions
- pulpcapping and pulpotomy

In the root:

- repair of root and furcation perforations

- repair of perforating internal resorptions
- repair of external resorption apexification
- root-end filling in endodontic surgery (retrograde filling)

Components

Biodentine is manufactured in single dose applications consisting of a powdered capsule and separate single-dose container of liquid (Koubi et al., 2013). The powder is principally composed of core materials, tri-calcium silicate and di-calcium silicate. (Nowiscka et al., 2013). Calcium carbonate and oxide are added fillers that have been shown to act as a nucleation site, enhancing the microstructure (Grech, Mallia, & Camilleri, 2013). Iron oxide and zirconium oxide (radiopacifier) also contribute to the powder of Biodentine (Han & Okiji, 2013). The liquid component consists of calcium chloride, which is used as a setting accelerator and water-reducing agent in aqueous solution with an admixture of polycarboxylate (a superplasticizing agent) (Nowiscka et al., 2013).

Setting Reaction

To prepare Biodentine, 5 drops of the liquid are added to the powder within the plastic capsule. The capsule needs to be mechanically mixed for 30 seconds at a speed of 4000-4200 rotations/min using a device such as an amalgamator (Kayahan, et al., 2013).

The hydration of the tricalcium silicate ($3\text{CaO} \cdot \text{SiO}_2 + \text{C}_3\text{S}$) produces a hydrated calcium silicate gel (CSH gel) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) (Camilleri et al., 2014).



C₃S

CSH

The dissolution process occurs at the surface of each grain of calcium silicate. The hydrated calcium silicate gel and the excess of calcium hydroxide tend to precipitate at the surface of the particles and in the pores of the powder, due to saturation of the medium (<http://www.septodontusa.com/>).

The consistency of Biodentine is similar to that of phosphate cement (Nowicka et al., 2013). The working properties of Biodentine have been described to be better than MTA, which was described to be grainy and sandy, and similar to intermediate restorative material (IRM) (Butt et al., 2014). When placed in simulated body fluid, Biodentine, in addition to other cements such as MTA, have demonstrated the deposition of hydroxyapatite on their surfaces (Camilleri, Sorrentino, & Damidot, 2013).

Setting Time

Biodentine can be applied directly to the tooth structure without requiring any surface treatment such as acid etching or a bonding system (Koubi et al., 2013).

The working time of Biodentine is up to 6 minutes with an initial set at around 10-12 minutes. However, a previous study found the final set time of Biodentine to be closer to 45 minutes (Grech et. al., 2013). Use of a rubber dam is essential, as water and saliva can prolong the initial setting time (Septodont, <http://www.septodontusa.com/>).

Restorative Clinical Applications

When placing an immediate enamel restoration (i.e. composite restoration) Biodentine should be placed in the cavity preparation so that the volume of missing dentin is replaced. If a matrix is used, it should not be removed before the 12 minute setting time. The permanent enamel restoration should be placed at the end of the setting time. If bonding to a composite resin restoration, a total etch or self etch adhesive system should be used.

It is important to point out that Biodentine can be used when placing an immediate enamel restoration (i.e., sandwich technique, pulp capping) and as a non-immediate enamel restoration. When used as a non-immediate restoration, the occlusal portion of the restoration should be removed within 1 week to 6 months and a permanent restoration (amalgam, composite, etc.) is to be placed (Septodont, <http://www.septodontusa.com/>). According to Koubi et al., Biodentine is acceptable as a posterior restoration for up to 6 months (Koubi et al., 2013). The compressive strength of Biodentine increases with time and will be strong enough to withstand chewing after a few hours.

Pulp Capping

Vital pulp therapy (direct pulp capping and pulpotomy) aims to treat reversible pulpal injury whenever the pulp is exposed by caries, trauma or mechanical (iatrogenic) processes. The ultimate goals of direct pulp capping are to maintain pulp vitality and function, and to completely restore dentin continuity beneath the injury (dentin bridging) (Tziafa et. al., 2014). Maintenance of pulp vitality is especially important in young patients with incomplete apical closure.

Biodentine has been indicated for use in direct pulp capping of mechanical pulp exposures as well as traumatic pulp exposures (broken tooth). Bajic found favorable therapeutic effects of Biodentine when used in direct pulp capping on Vietnamese pigs (Bajic et al., 2014). Nowicka found no significant differences between the dentinal bridge formation of Biodentine and MTA when used as a direct pulp cap in intact human molar teeth (Nowiscka et al., 2013).

Compressive Strength

If a material is placed under a load that tends to compress or shorten it, the internal resistance to such a load is considered a compressive stress.

Compressive strength, therefore, is the maximum compressive stress a material can sustain before breaking. Compressive strength is considered one of the main physical characteristics of hydraulic cements (Malkondu, Kazandag, & Kazazoglu, 2014). Permanent restorative materials, whether used in vital pulp therapy, the sandwich technique, or as a final restoration must have the capacity

and strength to withstand masticatory forces (Kayahan et al., 2013), as most masticatory forces are compressive in nature.

The International Organization for Standardization (ISO) was established in order to standardize testing protocols (Wang, D'Alpino, Lopes, & Pereira, 2003). The American Dental Association (ADA) also has standards, technical specifications and technical reports for dental products. Compressive strength is determined according to ISO 9917:2010 and ADA specification 96 for water based cements. ADA specification 96 specifies requirements and test methods for powder/liquid acid-base dental cements intended for permanent cementation, lining and restoration (www.ada.org).

To test compressive strength of a material, axial forces are applied towards each other, compressing the sample until it breaks. ISO 9917-1:2003 requires samples be cylindrical-shaped and have the dimension of 6 ± 0.1 mm in length and 4 ± 0.1 mm in diameter (Kayahan et al., 2013). If the proportion of the sample exceeds 2:1, it can result in undesirable bending of the specimen (Wang, D'Alpino, Lopes, & Pereira, 2003).

According to its manufacturer, one of the advantages of Biodentine compared to MTA and glass ionomer is that its compressive strength continues to improve with time over several days until it reaches a compressive strength similar to that of natural dentin (<http://www.septodontusa.com/>). Septodont attributes this maturation process to the low water/cement ratio which leads to a decrease in porosity over time. In a study by Grech, Biodentine was shown to

exhibit superior compressive strength and micro-hardness as compared to IRM, Bioaggregate, and tricalcium silicate cement replaced with 20% zirconium oxide (Grech et al., 2013). Biodentine has also been shown to exhibit significantly higher compression strength than MTA-Angelus (Butt et al., 2014).

The Sandwich Technique

The sandwich technique was first described by McLean and Wilson in 1977 using GIC as a base layer under a composite restoration (McLean & Wilson, 1977). With an open sandwich technique, a base layer of GIC is left exposed at the cervical margin to allow the release of fluoride to protect the surrounding tooth structure. In the closed sandwich technique, the GIC is applied to a cavity where complete enamel margins are available for bonding and sealing using the phosphoric acid etch technique (Sawani et. al., 2014). Resin modified glass ionomer cement (RMGIC) and flowable composite are also commonly used under composite in the sandwich technique.

Although calcium silicate based cements, such as the mineral trioxide aggregate (MTA), have excellent biological properties they are not frequently used in restorative dentistry due to their long setting times (Koubi et. al., 2012). However, Biodentine is specifically marketed as dentin replacement material in restorative dentistry (Malkondu, Kazandag, & Kazazoglu, 2014). Biodentine is a good candidate for a dentin substitute in open sandwich restorations for several reasons: it does not require photoactivation and thus can be placed in bulk in the

cavity. In addition, the manufacturer's setting time is short enough to complete the whole procedure in a single appointment and its mechanical properties are sufficient to withstand occlusal loading when protected with composite. In research conducted by Koubi, Biodentine performed as well as the RMGIC in open sandwich restorations (Koubi et. al., 2012).

Acid Etching

Conditioning the tooth prior to restoration with resin based materials is a common practice in modern adhesive dentistry. Phosphoric acid (35-40%) is one of the most common acid etchants of enamel and dentin. Acid etching removes the smear layer and provides a rough surface for the application of a bonding agent (Zafar & Ahmed, 2015). The loss of minerals and dissolution of hydroxapatite allows the hydrophilic bonding agent to infiltrate and create resin microtags, which micromechanically lock to the dental hard tissue.

An application of 15-30 seconds is the usual manufacturer recommendation for phosphoric acid etchant (Zafar & Ahmed, 2015), however, certain clinical conditions such as fluorosis, require longer etching times. However, etching for longer than 30 seconds is likely to increase the surface roughness and decrease surface hardness thus having a deleterious effect on the overall bond strength (Zafar & Ahmed, 2015).

Acid etching is a key step following the application of Biodentine when adhesively bonding a restoration, such as composite resin. However, if the

application of phosphoric acid etch significantly alters the physical properties of a material that is used as a pulp capping agent or as a base in the sandwich technique, such as Biodentine, the choice of restorative material may be limited to an unbonded restoration.

Hypothesis

Phosphoric acid etch procedures will not reduce the compressive strength of Biodentine.

Materials and Methods

Compressive strength of Biodentine was determined according to ISO 9917:2010 and ADA specification No. 96 for water based cements. Specimens were prepared using stainless steel cylindrical split molds (Sabri Dental Enterprises Inc, Downers Grove, IL) see Figure 1. with the internal dimension of 4.0 ± 0.1 mm in diameter and 6 ± 0.1 mm in length. Biodentine was prepared per manufacturer's recommended instructions and, using the plastic instrument provided by Septodont, condensed in to the cylindrical molds. Specimens were randomly divided into two groups—phosphoric acid etched Biodentine and non-etched Biodentine.

Attempts were made to remove all air bubbles. The specimens were stored in a Heratherm incubator (Thermo Fisher Scientific, Waltham, MA) for 15

minutes in 100% humidity at 37 degrees Celsius and then removed from the mold. If the specimens had air bubbles, they were discarded and a new sample was prepared. The ends of all the samples were polished using super fine grit sandpaper (3M, St. Paul, MN). The acid-etched samples were then subjected to 40% phosphoric acid etch(Henry Schein, Melville, IL) for 15 seconds, rinsed, and dried before testing.

The specimens were placed at a 90 degree angle and centered on the platen of the Universal Testing Machine (Instron 5943, Norwood, MA) see Figure 2. The specimens were then loaded to failure in compression using a cross head speed of 30mm/min. Twenty specimens from each group were tested.

$$\sigma_{cs} = \frac{4F}{\pi d^2}$$

The above equation was used to calculate the compressive strength in MPa. Where F is the force applied just before failure and d is the diameter. The compressive strength and standard deviation were calculated for each set.

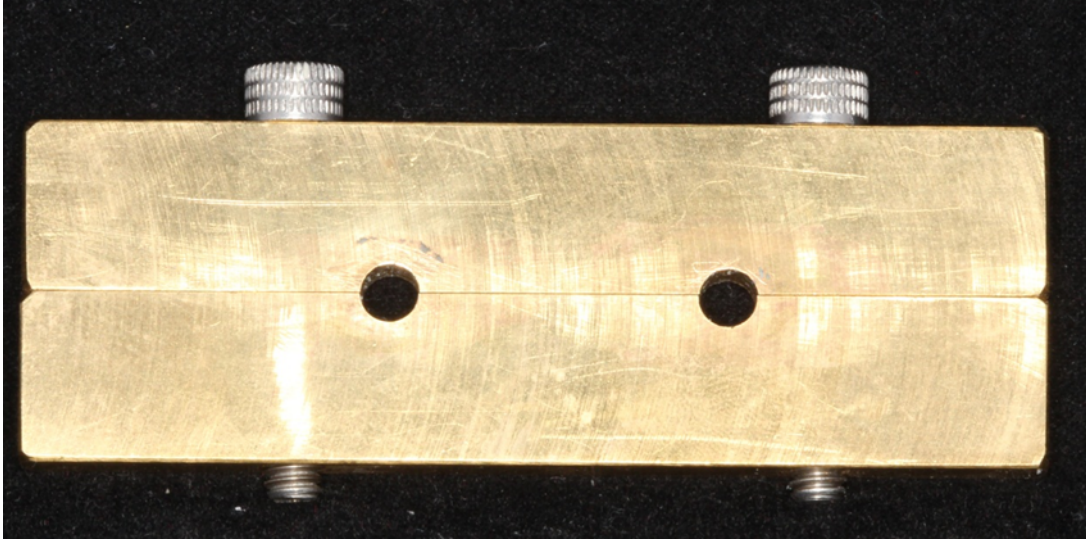


Figure 1. Stainless steel cylindrical split molds (Sabri Downers Grove IL) with the internal dimension of 4.0 ± 0.1 mm in diameter and 6 ± 0.1 mm in length used to prepare Biodentine samples into uniform dimension.



Figure 2. Photograph of the Instron 5943 used in testing samples of Biodentine under compression

Results and Statistical Analysis

In order to compare the compressive stress at maximum load (MPa) and the maximum load (lbf), independent sample t-tests were run. These tests were found to be statistically non-significant, $t(38) = .30$, $p = 0.77$; and $t(38) = .29$, $p = 0.77$ respectively. These results indicate that the acid-etched sample ($M = 2.62$, $SD = 0.70$) was not significantly different than the non-etched sample ($M = 2.68$, $SD = 0.70$) in terms of the compressive stress experienced at maximum load. Additionally, results indicate that the acid-etched sample ($M = 7.39$, $SD = 1.98$) was not significantly different than the non-etched sample ($M = 7.57$, $SD = 1.98$) in terms of the maximum load.

The output is listed below with short explanations for each:

Table 1:
The mean and standard deviations for the two groups are listed below.

Group Statistics					
Group		N	Mean	Std. Deviation	Std. Error Mean
Compressive stress at Maximum Load (MPa)	non-etched	20	2.68	.70	.16
	acid-etched	20	2.62	.70	.16
Maximum Load (lbf)	non-etched	20	7.57	1.98	.44
	acid-etched	20	7.39	1.98	.44

Table 2:

This table shows that the data come from a normal distribution (thus meeting the assumption (requirement) of the independent samples t-test)

Tests of Normality				
Group		Shapiro-Wilk		
		Statistic	df	Sig.
Compressive stress at Maximum Load (MPa)	non-etched	.971	20	.783
	acid-etched	.977	20	.889
Maximum Load (lbf)	non-etched	.971	20	.783
	acid-etched	.977	20	.889

Table 3:

This table indicates that the variance between the two groups was not statistically significant

Test of Homogeneity of Variance					
		Levene Statistic	df1	df2	Sig.
Compressive stress at Maximum Load (MPa)	Based on Mean	.030	1	38	.863
	Based on Median	.053	1	38	.818
	Based on Median and with adjusted df	.053	1	37.413	.818
	Based on trimmed mean	.041	1	38	.841
Maximum Load (lbf)	Based on Mean	.030	1	38	.864
	Based on Median	.053	1	38	.819
	Based on Median and with adjusted df	.053	1	37.412	.819
	Based on trimmed mean	.040	1	38	.842

Table 4:

This table fails to show a significant difference between the two groups

Independent Samples Test								
		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Compressive stress at Maximum Load (MPa)	Equal variances assumed	.295	38	.77	.07	.22	-.38	.51
Maximum Load (lbf)	Equal variances assumed	.294	38	.77	.18	.63	-1.09	1.45

Discussion

The null hypothesis was confirmed. No significant differences were found between the test group (acid etched) and the control group (non-acid etched) at the time point of 15 minutes. Its manufacturer claims that the working time of Biodentine is 6 minutes with a final set at around 10-12 minutes (Septodont, <http://www.septodontusa.com/>). Therefore, after a time period of 15 minutes, Biodentine is then ready to be placed as an immediate or non-immediate enamel restoration.

The ideal material for a pulp capping and base in the sandwich technique would have a compressive strength similar to that of dentin (297 MPa) or the composite resin restoration that would be placed over it. In addition, the material should set quickly enough that the final restoration (in this case, composite) could be placed immediately (Craig & Peyton, 1958).

In this study, the average compressive strength of the Biodentine was 2.680 MPa for the control group and 2.615 MPa for the group subjected to a phosphoric acid etch, which is significantly lower than the compressive strength of dentin. These results question Biodentine's ability to support an immediate enamel restoration—such as composite resin.

The results from this study are supported by a recent study performed by Nielson et. al. in which the compressive strength of Biodentine was calculated after 15 minutes, 3 hours, and 24 hours. At 15 minutes the average compressive strength in their study was only 1.3 MPa. At 24 hours, the average compressive

strength was 40.1 MPa which is significantly lower than the manufacturer's claim of 241.1 MPa (Nielsen et. al., 2016). The results from this study are also supported by another recent study performed by Kayahan et. al., in which the compressive strength of phosphoric acid etched Biodentine was measured at one week. They found that acid etching procedures had no adverse effects on the compressive strength of Biodentine or ProRoot MTA (Kayahan et al., 2013).

Conclusion

After a time period of 15 minutes, a 40% phosphoric acid etch procedure had no adverse effect on the compressive strength of Biodentine. However, the initial low compressive strength of Biodentine must be taken into consideration when it is used as an indirect pulp cap, as part of the sandwich technique, or even as a temporary restoration. Additional studies are needed to calculate the compressive strength of acid etched Biodentine at different time points to determine the ideal time point a permanent restoration should be placed.

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Appendix: Raw Data Results

Control (Non-Acid Etched)

	Compressive stress at Maximum Load (MPa)	Diameter (mm)	Maximum Load (lbf)
1	2.201	4.000	6.21651
2	2.688	4.000	7.59266
3	2.972	4.000	8.39486
4	2.119	4.000	5.98574
5	1.952	4.000	5.51563
6	2.499	4.000	7.06109
7	2.022	4.000	5.71133
8	3.205	4.000	9.05402
9	1.697	4.000	4.79385
10	2.910	4.000	8.21955
11	3.638	4.000	10.27620
12	3.432	4.000	9.69569
13	1.871	4.000	5.28511
14	3.222	4.000	9.10198
15	2.746	4.000	7.75622
16	1.566	4.000	4.42408
17	2.837	4.000	8.01567
18	2.407	4.000	6.80009
19	3.620	4.000	10.22557
20	4.008	4.000	11.32381
Mean	2.680	4.000	7.57248
Standard Distribution	0.70123	0.00000	1.98101
Maximum	4.008	4.000	11.32381

Test Group (Acid-Etched)

	Compressive stress at Maximum Load (MPa)	Diameter (mm)	Maximum Load (lbf)
1	2.643	4.000	7.46569
2	3.357	4.000	9.48369
3	1.895	4.000	5.35288
4	3.059	4.000	8.64254
5	3.823	4.000	10.80095
6	2.054	4.000	5.80349
7	2.737	4.000	7.73079
8	3.490	4.000	9.85998
9	2.470	4.000	6.97803
10	2.675	4.000	7.55812
11	2.727	4.000	7.70490
12	1.192	4.000	3.36706
13	2.138	4.000	6.03928
14	1.661	4.000	4.69194
15	2.708	4.000	7.65088
16	3.327	4.000	9.39863
17	2.955	4.000	8.34848
18	2.243	4.000	6.33780
19	3.460	4.000	9.77519
20	1.691	4.000	4.77773
Mean	2.615	4.000	7.38840
Standard Distribution	0.70189	0.00000	1.98286
Maximum	3.823	4.000	10.80095

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